I. AMENDMENT

IN THE SPECIFICATION

- Please amend the paragraph beginning at page 3, line 10, as follows:

-- Although the inductive phase of T cell help is Ag-dependent and MHC-restricted (Janeway et al, *Immun. Rev.*, 101:34 (1988); Katz et al, *Proc. Natl. Acad. Sci., USA*, 10:2624 (1973); Zinkernagle, *Adv. Exp. Med. Biol.*, 66:527 (1976)); the effector phase of T cell helper function can be Ag-independent and MHC-nonrestricted (Clement et al, *J. Immunol.*, 132:740 (1984); Hirohata et al, *J. Immunol.*, 140:3736 (1988); ~~Whalen~~ Bartlett et al, *J. Immunol.*, 143:~~1715~~ 1745 (~~1988~~ 1989)). An additional contrasting feature is that the inductive phase of T cell help often requires CD4 molecules and is inhibited by anti-CD4 mAb (Rogozinski et al, *J. Immunol.*, 126:735 (1984)), whereas helper effector function does not require CD4 molecules (Friedman et al, *Cell Immunol.*, 103:105 (1986)) and is not inhibited by anti-CD4 mAbs (Brian, *Proc. Natl. Acad. Sci., USA*, 85:564 (1988); Hirohata et al, *J. Immunol.*, 140:3736 (1988); ~~Whalen~~ Bartlett et al, *J. Immunol.*, 143:1745 (~~1988~~ 1989); and Tohma et al, *J. Immunol.*, 146:2547 (1991)). The non-specific helper effector function is believed to be focused on specific B cell targets by the localized nature of the T-B cell interactions with antigen specific, cognate pairs (Bartlett et al, *J. Immunol.*, 143:1745 (1989); Kupfer et al, *J. Exp. Med.*, 165:1565 (1987) and Poo et al, *Nature*, 332:378 (1988)). --

- Please amend the five paragraphs describing figures 4-8 beginning at page 19, line 11, as follows:

-- **Figure 4** [SEQ ID NO:~~24~~ 44] contains the amino acid sequence and DNA sequence corresponding to a preferred humanized variable light sequence (including the complementarity determining regions) referred to as VL#1 or preferred humanized variable light sequence (1).

Figure 5 [SEQ ID NO:~~25~~ 46] contains the amino acid and DNA sequence corresponding to a preferred humanized variable ligand sequence (including the complementarity determining regions) referred to as VL#2 or preferred humanized variable light sequence (2).

Figure 6 [SEQ ID NO:~~26~~ 48] contains the amino acid and DNA sequence corresponding to a preferred humanized variable heavy sequence (including the complementarity determining regions) referred to as VH#1 of preferred humanized variable heavy sequence (1).

Figure 7 [SEQ ID NO:~~27~~ 50] contains the amino acid and DNA sequence of the variable light sequence of 24-31 (non-humanized).

Figure 8 [SEQ ID NO:~~28~~ 52] contains the amino acid and DNA sequence of the variable heavy sequence of 24-31 (non-humanized). - -

- Please amend the paragraph beginning at page 25, line 4, as follows:

- - The cloning of the variable regions of 24-31 (described in detail in the examples *infra*) resulted in the identification of the V_L and V_H and sequences utilized by the 24-31 antibody respectively shown in **Figure 7** [SEQ ID NO:~~27~~ 50] and **Figure 8** [SEQ ID NO:~~28~~ 52]. After sequencing, the variable regions were then humanized. As noted, this was effected substantially according to the method of Padlan (1994) (Id.), incorporated by reference *supra*. - -

- Please amend the paragraph beginning at page 25, line 15, as follows:

- - More specifically, the 24-31 V_K and V_H sequences set forth in **Figure 7** [SEQ ID NO:~~27~~ 50] and **Figure 8** [SEQ ID NO:~~28~~ 52] were humanized by comparison to human antibodies of reported sequence, which are referred to as "templates." - -

- Please amend the paragraph beginning at page 26, line 7, as follows:

- - This methodology resulted in the following preferred humanized V_L and V_H heavy sequences derived from the 24-31 antibody which are set forth below in **Table 1** [SEQ ID NOs:1-4 and 9] and **Table 2** [SEQ ID NOs:5-8 and 10]. As discussed above, the invention further embraces equivalents and variants of these preferred humanized sequences, e.g., those containing one or more conservative amino acid substitutions which do not substantially affect gp39 binding. The complementarity determining regions are identified in **Figure 7**

[SEQ ID NO:~~27~~ 50] and Figure 8 [SEQ ID NO:~~28~~ 52] which contain the entire variable heavy and light chain CDR sequences of the parent (non-humanized) 24-31 antibody. - -

- Please amend TABLE 1 beginning at page 27, line 1, as follows:

- -

TABLE 1

HUMANIZED 24-31 VL SEQUENCES

~~10 20 40 60 70 80~~

24-31 DIVMTQSQKFMSTSVGDRVSITC KASQNVITAVA WYQQKPGQSPKLLIY SASNRYT

~~100~~

GVPDRFSGSGSGTDFTLTISNMQSEDLADYFC QQYNSYPYT FGGGTKLEIK [SEQ ID NO: 9]

(1) DIVMTQSPSFLSASVGDRVTITC KASQNVITAVA WYQQKPGKSPKLLIY SASNRYT GVPDRFSGSGSGTDFTLTISSLQPEDFADYFC QQYNSYPYT FGGGTKLEIK [SEQ ID NO: 1]

(2) DIVMTQSPDSLAVSLGERATINC KASQNVITAVA WYQQKPGQSPKLLIY SASNRYT GVPDRFSGSGSGTDFTLTISSLQAEDVADYFC QQYNSYPYT FGGGTKLEIK [SEQ ID NO: 2]

(3) DIVMTQSPSFMSTSVGDRVTITC KASQNVITAVA WYQQKPGKSPKLLIY SASNRT GVPDRFSGSGSGTDFTLTISSMQPEDFADYFC QQYNSYPYT FGGGTKLEIK [SEQ ID NO: 3]

(4) DIVMTQSPDSMATSLGERVTINC KASQNVITAVA WYQQKPGQSPKLLIY SASNRYT GVPDRFSGSGSGTDFTLTISSMQAEDVADYFC QQYNSYPYT FGGGTKLEIK [SEQ ID NO: 4] - -

- Please amend TABLE 2 beginning at page 27, line 31, as follows:

- -

TABLE 2

HUMANIZED 24-31 VH SEQUENCES

~~10 20 30 40 70 82abc 90~~

24-31 EVQLQESGPSLVKPSQTLSLTCSVTGDSIT NGFWI WIRKFPGNKLEYMG YISYSGSTYYNPSLKS RISITRDTSQNQFYLQLNSVTTEDTGTYYCAC

~~110~~

RSYGRTPYYFDF WGQGTTLTVSS [SEQ ID NO: 10]

(1) EVQLQESGPGLVKPSETLSLTCTVSGDSIT NGFWI WIRKPPGNKLEYMG
YISYSGSTYYNPSLKS RISISRDTSKNQFSLKLSSVTAADTGVYYCAC
RSYGRTPYYFDF WGQGTTLTVSS [SEQ ID NO: 5]

(2) EVQLQESGPGLVKPSQTLSLTCTVSGDSIT NGFWI WIRKHPGNKLEYMG
YISYSGSTYYNPSLKS RISISRDTSKNQFSLKLSSVTAADTGVYYCAC
RSYGRTPYYFDF WGQGTTLTVSS [SEQ ID NO: 6]

(3) EVQLQESGPGLVKPSQTLSLTCAVSGDSIT NGFWI WIRKHPGNKLEYMG
YISYSGSTYYNPSLKS RISISRDTSNNQFSLNLNSVTRADTGVYYCAC
RSYGRTPYYFDF WGQGTTLTVSS [SEQ ID NO: 7]

(4) EVQLQESGPGLVKPSETLSLTCAVYGDSIT NGFWI WIRKPPGNKLEYMG
YISYSGSTYYNPSLKS RISISRDTSKNQFYLKLSSVTAADTGVYYCAC
RSYGRTPYYFDF WGQGTTLTVSS [SEQ ID NO: 8] - -

- Please amend the paragraph beginning at page 29, line 7, as follows:

-- So as to better describe the subject humanized 24-31, V_H and V_L sequences, the preferred humanized framework sequences [SEQ ID NOS:. ~~9-21~~ 11-41] are also set forth in **Table 3** below, which compares these sequences to the template human variable heavy and light framework sequences, i.e., human DEN VK1, Human o12/V36 germline, human LEN VKIV, human 58p2, human Z18320, and human 3d75d as well as to the unhumanized murine 24-31 V_H and V_L framework sequences. - -

- Please amend TABLE 3 beginning at page 30, line 1, as follows:

- -

TABLE 3

VK Framework Region Comparisons - Humanized Anti-gp39

	FR1	FR2
Human 012/V3b germline [SEQ ID NO: 11]	DIQMTQSPSFLSASVGDRVTITC	WYQQKPGKAPKLLIY
Human DEN VKI [SEQ ID NO: 12]	---------T----------	--------E---V---
Murine 24-31 [SEQ ID NO: 13]	--V----QK-M-T------S---	----.---QS------
Padlan VL#1 humanized [SEQ ID NO: 14]	--V----------	--------S------

	FR3	FR4	
Human 012/V3b	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	FGQGTKLEIK	[SEQ ID NO: 15]
Human DEN VK1	----------E---------SD--------		[SEQ ID NO: 16]
Murine 24-31	---D----------NM-SE-L-D-F- --G-------		[SEQ ID NO: 17]
Padlan VL#1	---D----------D-F- --G-------		[SEQ ID NO: 18]

TABLE 3 (Continued)

	FR1	FR2	
Human LEN VKIV	DIVMTQSPDSLAVSLGERATINC	WYQQKPGQPPKLLIY	[SEQ ID NO: 19]
Murine 24-31	-------QKFMST-V-D-VS-T-	--------S------	[SEQ ID NO: 20]
Padlan VL#2 humanized	----------	--------S------	[SEQ ID NO: 21]

	FR3	FR4	
Human LEN VKIV	GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC	FGQGTKLEIK	[SEQ ID NO: 22]
Murine 24-31	----------NM-S--L-D-F- --G-------		[SEQ ID NO: 23]
Padlan VL#2	----------D-F- --G-------		[SEQ ID NO: 24]

	FR1	
Human 58p2	QVQLQESGPGLVKPSETLSLTCTVSGGSIS	[SEQ ID NO: 25]
Murine 24-31	E---------S----Q------S-T-D--T	[SEQ ID NO: 26]
Padlan VH#1 humanized	E----------D--T	[SEQ ID NO: 27]
Human Z18320 GenBank	----------Q----------	[SEQ ID NO: 28]
Human 3d75d germline	----------Q----------	[SEQ ID NO: 29]
Padlan VH#2 humanized	E----------Q----------D--T	[SEQ ID NO: 30]

FR2 FR3

Human 58p2 [SEQ ID NO: 31]	WIRQPPGKGLEWIG RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR
Murine 24-31 [SEQ ID NO: 32]	---KF--NK--YM- -IS-TR---Q---Y-Q-N---TE--GT----C
Padlan VH#1 [SEQ ID NO: 33]	---K---NK--YM- -IS--R------------------G-----C
Human Z18320 [SEQ ID NO: 34]	-----A-------- ----------
Human 3d75d [SEQ ID NO: 35]	----H--------- ----------
Padlan VH#2 [SEQ ID NO: 36]	---KH--NK--YM- -IS--R------------------G-----C
Human 58p2	WGQGTMVTVSS [SEQ ID NO: 37]
Murine 24-31	-----TL---- [SEQ ID NO: 38]
Padlan VH#1	-----TL---- [SEQ ID NO: 39]
Human Z18320	---------- [SEQ ID NO: 40]
Padlan VH#2	-----TL---- [SEQ ID NO: 41] - -

- Please amend the paragraph beginning at page 32, line 6, as follows:

-- In order to produce humanized antibodies, DNA sequences are synthesized which encode for the afore-identified humanized V_L and V_H sequences. As noted, taking into account these four humanized V_L sequences, and four humanized V_H sequences, there are 16 potential humanized antigen combining sites which may be synthesized. Also, there are even more potential humanized antigen combining sites taking into account the potential substitution of residues 34, 43, 44 and 68 of the humanized V_H and residue 85 of the humanized V_L by other amino acid residues and/or the potential incorporation of conservative substitution mutations. Two of the preferred humanized variable light sequences (1) and (2) and a preferred humanized variable heavy sequence (1) including the complementarity determining regions and corresponding DNA sequences are set forth in **Figures 4, 5, and 6** [SEQ ID NOS: ~~24-26~~ 44, 46, and 48], respectively. --

- Please amend the paragraph beginning at page 33, line 27, as follows:

-- Preferred modifications of the human gamma 4 constant domain include ~~P~~ E and/or ~~E~~ P modifications, which respectively refer to the change of a leucine to a glutamic acid at position 236 and/or the change of a serine to a proline ~~(Kabat numbering)~~ at position 229 such as described in commonly assigned ~~Attorney Docket No. 012712-165~~ U.S. Patent Application No. 08/523,894, filed on September 6, 1995, which issued as U.S. Patent No. 6,136,310 on October 24, 2000, and incorporated by reference in its entirety herein. --

- Please amend the paragraph beginning at page 44, line 9, as follows:

-- **a. Preparation of cDNA.** PolyA$^+$ mRNA was prepared from 2 x 10^6 cells each of the 24-31 hybridoma and the NS1 cell line, (Carroll et al, Mol. Immunol., 10:991 (1988)), the fusion partner used in the generation of the 24-31 hybridoma, utilizing an Invitrogen Corporation MicroFast Track™ mRNA isolation kit, according to the manufacturer's protocol. First strand cDNA was synthesized utilizing 50 pmoles oligo-dT and 5 units M-MLV reverse transcriptase (Promega) (Sambrook et al, Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press (1989)) followed by Sephadex G-25 chromatography. --

- Please amend the paragraph beginning at page 44, line 18, as follows:

-- **b. PCR amplification of V_k and V_H cDNA.** 24-31 and NS1 cDNA were amplified by PCR using a panel of 5' primers specific for V_k or V_H leader sequences in combination with 3' constant region primers. The panel of 5' V_H primers are identical to those described by Jones and Bendig (Bio/Technol., 9:88 (1991); Errata, Bio/Technol., 9:579 (1991)). The panel of 5' V_k primers (Jones et al., (id.)) were modified to convert the Sal I cloning site recognition sequences (GTCGAC) into Bgl II recognition sequences (AGATCT) to facilitate the cloning of the amplified gene segments into IDEC's N5KG1 expression vector (See **Figure 1**). The 3' V_k and V_H primers contain a Bsi WI cloning site sequence at amino acid positions 108-109 (numbering according to Kabat et al., "Sequences of Proteins of Immunological Interest," 5th Ed., NIH (1991)) and a Nhe I cloning site sequence at positions 114-115, respectively, and have the following sequences:

TGCAGCATCCGTACGTTTGATTCCAGCTT (C_k) [SEQ ID NO:~~22~~ 42] and

GGGGGGTGCGTGCTAGCTG (A/C) (G/A) GAGAC (G/A) GTGA (Cγ1) [SEQ ID NO:~~23~~ 43]. This primer panel has been previously used by the Assignee to amplify and clone the C2B8 anti-CD20 antibody (Nishioka et al., J. Immunol., 153:1027 (1994)) and numerous other mouse V_k and V_H gene segments (data not shown). - -

- Please amend the paragraph beginning at page 48, line 26, as follows:

- - After establishing that humanized anti-gp39 binds to gp39, an assay is effected to confirm that the humanized anti-gp39 retains its ability to block the binding of the ligand to its receptor. For this purpose, activated human peripheral blood T cells, or the gp39-transfected CHO cells, 50D4, are pretreated with graded concentrations of murine 24-31 or with humanized 24-31 for 15 minutes at ~~4EC~~ 4°C. Following this preincubation, CD40-Ig-biotin is added and the binding determined by flow cytometry using PE-avidin. Concentrations of mAbs to achieve a 50% reduction in CD40-Ig binding are determined. - -

- Please amend the paragraph beginning at page 50, line 5, as follows:

- - 5. Flick plate and blot to remove excess glutaraldehyde. Add 150 µl/well of 100 mM glycine with 0.1% BSA and incubate for 30 minutes at room temperature. Plates can be used immediately or frozen at ~~-20EC~~ -20°C for future use. - -

- Please amend the paragraph beginning at page 50, line 31, as follows:

- - The ability of the chimeric and humanized (version 1) 24-31 antibodies to compete with the murine 24-31 for binding to mgp39-CHO cells basis was evaluated. The ability of the humanized 24-31 to compete with the murine 24-31 for binding to mgp39-CHO was used to evaluate whether in the humanized antibody the exchanges of the murine framework residues with their human counterparts resulted in a significant loss (~~E 3x~~ ≥ 3x decrease) of affinity.

- Please amend the paragraph beginning at page 51, line 15, as follows:

- - 5. Flick plate and blot to remove excess glutaraldehyde. Add 150 µl/well of 100 mM glycine with 0.1% BSA and incubate for 30 minutes at room temperature. Plates can be used immediately or frozen at ~~-20EC~~ -20°C for future use. - -

- Please amend the paragraph beginning at page 52, line 22, as follows:

-- 2. Selectively purify T and B cells from a buffy coat using Lympho-Kwik reagents. Activate the T cells with 50 µg/ml mitomycin C per $5x10^6$ cells for 30 minutes at ~~37□C~~ 37°C. --

- Please amend the paragraph beginning at page 52, line 31, as follows:

-- 6. Incubate plate at ~~37□C/5%~~ 37°C/5% CO_2 for 12 days. --

- Please amend the paragraph beginning at page 54, line 1, as follows:

-- Direct binding of the ^{125}I-labeled antibody to murine gp39-CHO cells was tested in a dilution series, in order to determine both counts/µg and the appropriate working concentration (□ =half-maximal binding concentration). --

- Please amend the paragraph beginning at page 55, line 1, as follows:

-- The purified $CD4^+$ cells were activated by co-culturing with soluble anti-CD40L antibodies and immobilized antibody to CD3. Plastic flat-bottom tissue culture plates (Costar) were coated overnight at ~~4□C~~ 4°C with goat anti-mouse IG (10 µg/ml) in 100 and 500 µl volumes per well for 96 and 48 well plates, respectively. The next day, the plates were blocked with 5% FBS-RPMI 1640 for 1 hour at room temperature and then washed twice. Mouse anti-human CD3 mAb was subsequently added at concentrations of 1, 10, 100 and 1000 ng/mL, 100 µl for 96-well plates and 500 µl for 48-well plates. The plates were incubated with anti-CD3 overnight at ~~4□C~~ 4°C. The following day, the plates were washed twice with RPMI 1640 containing 5% FBS anti-human CD154 (CD40L) monoclonal antibodies TRAP1, IDEC131, and control human IgG1 were added in soluble form at concentrations ranging from 1-1000 ng/mL to each of the four plates containing various immobilized concentrations of anti-CD3 antibodies. Purified $CD4^+$ T cells suspended in 10% FBS-Iscove's medium were added at 5×10^4 cells/well in a volume of 200 µl in 96-well plates of 2.5×10^5 cells/well in a volume of 1 ml to 48-well plates and cultured for 48 hours at ~~37□C~~ 37°C in a 5% CO_2 incubator. After the 48-hour period, 100 µl aliquots of culture media were collected from each well and stored at ~~-70□C~~ -70°C in 96-well, round-bottom plates (Costar) for analysis of the various cytokines. --

- Please amend the paragraph beginning at page 65, line 8, as follows:

-- The polawax, beeswax and lanolin are heated together at ~~60□C~~ 60°C. A solution of methyl hydroxybenzoate is added and homogenization is achieved using high speed stirring. The temperature is then allowed to fall to ~~50□C~~ 50°C. The antibody or fragment thereof of the invention is then added and dispersed throughout, and the composition is allowed to cool with slow speed stirring. --

- Please amend the paragraph beginning at page 65, line 19, as follows:

-- The methyl hydroxybenzoate and glycerin are dissolved in 70 ml. of the water at ~~75□C~~ 75°C. The sorbitan monolaurate, polysorbate 20 and cetostearyl alcohol are melted together at ~~75□C~~ 75°C and added to the aqueous solution. The resulting emulsion is homogenized, allowed to cool with continuous stirring and the antibody or fragment thereof of the invention is added as a suspension in the remaining water. The whole suspension is stirred until homogenized. --

- Please amend the paragraph beginning at page 65, line 30, as follows:

-- The methyl and propyl hydroxybenzoates are dissolved in 70 ml. purified water at ~~75□C~~ 75°C and the resulting solution is allowed to cool. The antibody or fragment thereof of the invention is then added, and the solution is sterilized by filtration through a membrane filter (0.022 Am pore size), and packed aseptically into suitable sterile containers. --